STUDY OF BIOMOLECULE BINDING WITH DIFFERENT RECEPTOR SURFACES AND DOPING EFFECT ON CONJUGATED POLYMERS

by

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Abstract

This dissertation mainly includes two research themes. The first theme is related to biomolecules, specifically antibodies, bonding to receptor surfaces, in order to find stable and sensitive materials for immuno-biosensors. The receptor materials are oxidized graphene oxide (GO-COOH) and PT-COOH. GO-COOH is a derivative of graphene and PT-COOH is a kind of conjugated polymer. Electrical ELISA and fluorescence tests are done that indicates both can be good supports for antibodies. The second theme is the doping effect on conjugated polymers. P3HT and PT-COOH are both conjugated polymers and are tested for pH sensitivity after doping by the p-type dopant F4TCNQ. The pH sensitivity of conjugated polymers can come from ionic doping based on a prior experiment result, and the doping experiment helps prove the hypothesis.

Acknowledgements

I really appreciate my advisor professor Howard Katz, for helping me and guiding me in my research work, he is a really kind and instructive advisor and has gave me a lot of help. I am grateful to other professors who help me in my research work, such as professor Art Bragg and professor Peter Searson. I also appreciate Hyun-June Jang and other my fellow students, who also help me a lot in my research work.

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Introduction

1.1 Motivation

Biosensors have multiple types and wide application. The development of biosensors is essential because they can help diagnose diseases in advance. In this dissertation, the immuno-biosensor is mainly studied, which is a type of biosensor aimed at studying the interaction of antigen and antibody. There are multiple steps to fabricate a biosensor, including the immobilization of antibody, antigen-antibody interaction and detection of the antigen-antibody interaction. The aim of this dissertation is to find materials that can be used as a good support for the antibody, and at the same time make sure the materials are stable and sensitive as sensing layers. At the same time, pH sensitivity of conjugated polymers was discovered by our group, and to prove the hypothesis that the sensitivity is caused by ionic doping, pre-doping studies are necessary.

1.2 Antibody and coupling agent

Each antibody molecule is structurally composed of two identical heavy chains (HCs) and two identical light chains (LCs) assembled into three discrete functional domains. The most basic function of antibodies is to neutralize the targeted antigen. While the two antigen-binding fragments (Fabs) are responsible for binding to the

specific molecular target with high avidity, the crystallizable fragment (Fc) binds to immune receptors to elicit effector functions. [1] The antibody bonding to the immune receptors sometimes needs coupling agents. In this dissertation, the immune receptors are oxidized carbon oxide and the conjugated polymer PT-COOH A coupling agent, also called a crosslinking agent, is needed for the better antibody bonding with receptors since the receptors are not naturally designed for antibody bonding.

There are various classifications for the employed crosslinking agents. They can be classified on the basis of the type of bond formed, leading to covalent crosslinking, ionic bonds, and physical crosslinking created by hydrogen and Van der Waals bonds. In other classifications, crosslinkers are divided into three main types: physical, chemical, and enzymatic.[2] The conjugated polymers and oxidized carbon oxide have carboxylic acid groups, so it is better to have chemical conjugation between the primary amine groups of the antibody and the carboxyl groups; the formation of amide bonds is a good way to stabilize antibody on the receptor. Two main intermediates are considered as crosslinking agents where a carboxylic acid is involved: carbodiimides and aziridines. The general reaction involves the condensation of a carbodiimide reactant with a carboxylic acid, leading to an acetyl urea. The reaction for aziridines is that the carboxylic acid groups can be spontaneously led to an amino ester at room temperature. But there are some drawbacks for aziridines; they have lower water solubility and also higher toxicity than carbodiimide.[3] Since the carbodiimide crosslinker has better properties than the aziridine crosslinker, one kind of carbodiimide crosslinker is chosen to be used in the experiments. EDC/NHS (1-ethyl-3-(3-dimethylaminopropyl)

carbodiimide/N-hydroxysuccinimide) activation is a good choice for carboxylic acid coupling. EDC is a water-soluble carbodiimide used to couple carboxyl or phosphate groups to primary amines; it reacts with carboxyl groups. N-hydroxysuccinimide (NHS) or N-hydroxysulfoxuccinimide (sulfo-NHS) are usually added to the protocol to increase the EDC-mediated coupling efficiency. The presence of NHS or sulfo-NHS allows a two-step reaction that avoids intra and intermolecular cross-linking of the antibody, since this biomolecule contains both amine and carboxyl groups. EDC helps form amine-reactive intermediate, and NHS or sulfo-NHS converts this amine-reactive ester into a semi-stable active ester that reacts with the amine group on the antibody.[4]

1.3 Antibody immobilization detection

Antibody detection usually relies on the rection between antibody and antigen. There are multiple methods for antibody detection. Immunoprecipitation is an in vitro assay for the identification and semi quantification of soluble antibody. The addition of the antigen to the antibody results in the formation of a three-dimensional, insoluble network of precipitating aggregates which can be detected by, e.g., a nephelometer. The immunoblot or dot blot technique is similar to the immunoprecipitation assay. However, in immunoblotting the antigen-antibody reaction takes place on the solid phase. The assay utilizes membranes to bind antigens. Antigens are applied in small dots, and the membranes are dried. The membranes are treated with blocking buffer, and unbonded antibodies are washed away. Then, the membranes are incubated with a secondary antibody raised against the antibody of interest which is conjugated with an enzyme.

The formation of the antigen-antibody-secondary antibody complexes can be visualized by adding a substrate which will be converted by the secondary antibody-bound enzyme to yield a colored spot. The use of secondary antibody-bound enzymes represents the basic ELISA (enzyme-linked immune adsorbent assay) principle. [5] ELISA utilizes an enzyme system to show specific combination of an antigen with its antibody. The enzyme system of ELISA usually consists of enzyme which is labeled with a specific antibody or antigen and a chromogenic substrate which is added after antigen-antibody reaction.[6] ELISA can be direct or indirect. Direct ELISA means antigen immobilized on a solid phase is detected directly with labeled specific antibody. Indirect ELISA means unlabeled specific antibody is first bound to antigen immobilized on a solid phase, then the antigen is detected indirectly by labeled "second antibody".[7]

In this dissertation, electrical ELISA is used to detect the antigen-antibody reaction, which is efficient in on-spot diagnostics of wide-spreading and contagious infections, so that the immobilization of the antibody can be revealed. Electrical ELISA is based on the field effect transistor (FET). By using the catalyst reaction of the labeled enzyme, the biological signal could be efficiently determined through the electrical response in the FET.[8]

1.4 Graphene based materials

Graphene is monolayers of carbon atoms arranged in a honeycomb network, which is a giant aromatic macromolecule that conducts both electricity and heat well in two dimensions.[9] Graphene is a kind of material that can be chemically modified in

multiple ways. Graphite oxide is a layered material consisting of hydrophilic oxygenated graphene sheets (graphene oxide sheets) bearing oxygen functional groups on their basal planes and edges. Graphite oxide can undergo complete exfoliation in water, yielding colloidal suspensions of almost entirely individual graphene oxide sheets. [10] Graphene oxide can reach a higher oxidation degree, which is named as oxidized graphene oxide. It can provide high O/C ratio by adding more carboxylic acid group on graphene oxide sheet. [11]

1.5 Conjugated polymers

Conjugated polymers are made up from unsaturated building blocks such as arenes, olefins or acetylenes which are connected by single bonds that have some π-bond character to create large domains of delocalized, polarizable π-electrons. It is their "box" of mobile π-electrons which allows macromolecules to serve as chromophores and electrophores, that is they can interact with light to take-up, transport or store electrical charges.[12] Conjugated polymers are widely used in organic field effect transistors(OFETs). Controlling the thin film morphology, assisted by the molecular self-assembly of CPs, plays a key role in deciding the performance of organic electronic devices. Owing to the inherent quasi-1-dimensional nature of CPs, transport properties can be easily enhanced if the main-chain backbone can be oriented in a particular direction, which can lead to anisotropic charge transport. [13]

1.6 Doping on polymers

Molecular doping allows us to control the electrical properties of organic semiconductors and is a powerful means to improve the performance of a variety of devices such as organic light-emitting diode and field-effect transistors. Doping involves the addition of a molecule—a molecular dopant—to the semiconducting host material, which introduces polarons by electron transfer. Electron acceptor 2,3,5,6-tetrafluoro-7,7,8,8-tetracyanoquinodimethane (F4TCNQ) is able to form a dianion in a two-electron reduction. F4TCNQ dianions have been observed in charge-transfer salts and through photogeneration in F4TCNQ crystals. [14]

2. Experiment design and method

2.1 Attachment of oxidized graphene oxide to PMMA substrate

2.1.1 Fabrication of oxidized graphene oxide (GO-COOH)

The enrichment of carboxylic acid group in oxidized graphene oxide can help it become a good receptor of antibody, and the hydroxy groups can help form hydrogen bonds between the oxidized graphene oxide and the PMMA substrate, so it can be the sensing layer when PMMA is the substrate.

Graphene oxide (GO) was bought from Sigma Aldrich, which has a concentration of 2mg/ml, dispersion in water. NaOH (1.2 g) and chloroacetic acid (Cl-CH_2-COOH) (1.0 g) were added to the GO suspension and bath sonicated for 3h to convert the -OH groups to -COOH groups via conjugation of acetic acid moieties giving GO-COOH.[14]

A thick black-colored solution can be derived, which is the GO-COOH solution.

2.1.2 Attachment of GO-COOH

The method for attachment includes two steps, spin coating and annealing. PMMA substrate is directly bought and has ITO on the front side, which can help increase the conductivity and will be helpful in the electrical measurement.

The first step is spin coating. Enough GO-COOH aqueous solution was added to cover the whole area of the substrate, around 0.3ml. This was spin coated at 1500rpm for 1min, to distribute GO-COOH as evenly as possible.

The second step is annealing, to enhance the formation of hydrogen bonding and evaporate the water inside GO-COOH, for one hour at 100℃.

2.2 Antibody functionalization on GO-COOH

The first step is the activation of carboxylic acid groups of GO-COOH to couple with the amine group on the antibody. EDC/NHS aqueous solution is used as the coupling agent. The concentration of EDC is 10mg/ml and NHS is 3mg/ml. 0.1ml EDC/NHS solution was added on the top of GO-COOH attached to the PMMA substrate and left for 20mins for activation.

The second step is antibody functionalization. The antibody is Myelin Basic Protein (MBP) antibody, having a concentration of 1mg/ml. The antibody was diluted to the concentration of 100ug/ml by 1x PBS buffer solution. 0.05ml antibody solution was added on the top of the activated GO-COOH layer and left for 6 hours for the

antibody automatically bond to the GO-COOH layer.

The schematic image can be indicated through Fig.1

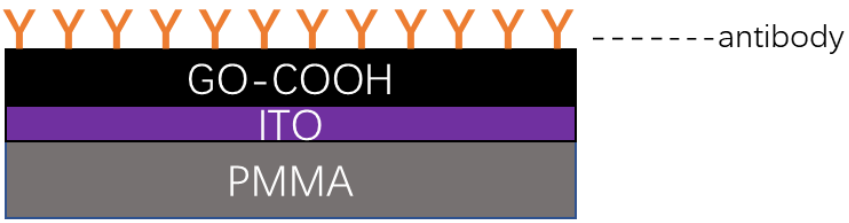


Fig 1

2.3 Antibody immobilization detection

ELISA is the method usually used for the detection of antibody-antigen reaction. Instead of using the conventional chemical detection method, electrical ELISA is used in this dissertation. The measurement system is called the remote gate sensing configuration, which means two different remote sensing gates were coupled to the gate of each commercial FET, and the sensing gates are connected with the antibody receptor substrate through Ag/AgCl electrodes. Two sensing signals can be given in every measurement. [15] The remote gate sensing configuration can help transfer the antibody-antigen reaction to electrical signal in a more efficient way. The system can be described in schematic image Fig.2.

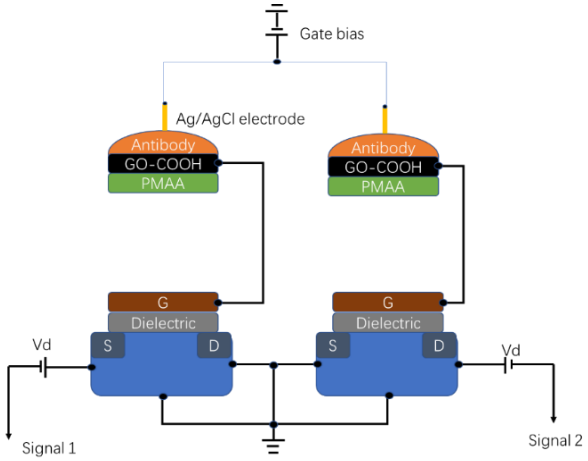


Fig 2.

2.3.1 pH test

The pH test is designed to measure the pH sensitivity of GO-COOH modified PMMA. pH3,4,6,7,9,10, or 11 buffer solution is added on the top of GO-COOH modified PMMA, graphene modified PMMA as well as neat PMMA. The change of PH sensitivity can be an evidence for the increasing concentration of carboxylic acid groups in GO-COOH, which can make it a good receptor for antibody immobilization.

2.3.2 Antigen test

Different concentrations of antigen reacting with their antibody on the receptors can change the surface potential of the films made by receptors of their antibody. The change of surface potential can be transferred to an electrical signal change during the electrical ELISA measurement. The concentrations of the antigen used, here MBP, are 100pg/ml, 1ng/ml, 10ng/ml, 100ng/ml, 1ug/ml and 10ug/ml. Then MBP is added on the surface of antibody-functionalized GO-COOH, and the ELISA measurement is started after the antigen is added.

2.4 Fabrication of conjugated polymer film

Poly [3-(3-carboxypropyl) thiophene-2,5-diyl] (PT-COOH) is used to make the conjugated polymer film. This polymer is used because it is a conjugated polymer, so it has conductivity itself as a sensing layer, and the carboxylic acid group can be used as receptor for antibody immobilization. The film making process requires two steps. The first step is making the PT-COOH solution. This polymer is dissolved in dimethylformamide (DMF), at 130℃ for 15mins. The concentration is 20mg/ml. The solution was filtered after cooling down.

The second step is spin-coating. The polymer film is made on silicon wafers. The silicon wafers are cleaned by piranha etch and UV ozone. After cleaning, the polymer solution is applied on the silicon wafer and spin coated for 320s at 1600rpm. If all the solvent DMF needs to be removed from the film, overnight anneal under 90℃ is required.

2.5 Antibody immobilization detection

2.5.1 Antibody immobilization

There are two methods for antibody immobilization. The first method is called the surface treatment method. Before the surface treatment method, the solvent in the polymer film needs to be removed. The first step is to activate the carboxylic acid group in the PT-COOH film. EDC/NHS is dissolved in ethanol with the concentration of 10mg/ml and 3mg/ml, respectively. The solution is obtained by EDC stirred in ethanol

for more than an hour and then NHS stirred overnight. The EDC/NHS solution is added on the top of PT-COOH film and left for 20mins. The polymer film was washed by 0.05x PBS solution, and then 10ug/ml MBP antibody was added on the activated polymer film and left for 3 hours.

The second method is the antibody embedded method. The EDC/NHS solution is made with the same process as the surface treatment method, but the solvent was changed to DMF instead of ethanol. The PT-COOH solution is made separately. The EDC/NHS solution is mixed with the PT-COOH solution together and stirred for an hour to activate the carboxylic acid groups in the polymer solution. 100ug/ml MBP antibody are then added into the activated solution and stirred for another two hours for antibody bonding to the receptors. The antibody-mixed solution will be spin coated on the silicon wafers for further test.

2.5.2 Antibody immobilization test

The first method for antibody-antigen reaction test is electrical ELISA measurement. The above configuration is used for the test. Different concentrations of MBP protein are added on the antibody functionalized film, from 100pg/ml to 10ug/ml.

The second method for testing is the fluorescence test. The detection antibody, FITC-MBP antibody, can have an absorbance fluorescent peak around 490nm, which can be used as the detection of the immobilization of the antibody in polymer film.

2.6 Doping effect on conjugated polymers

2.6.1 Doping on conjugated polymers

Poly(3-hexylthiophene) (P3HT) and PT-COOH film show a significant pH sensitivity, which is probably caused by the ionic doping during the pH sensitivity test. So, to prove the hypothesis, the polymer films are pre-doped before the pH test. P3HT and PT-COOH film are made under the similar process. The concentration of P3HT solution is 10mg/ml, and PT-COOH is 20mg/ml. The time for spin coating of P3HT is 60s instead of 320s. F4TCNQ is dissolved in DMF at the concentration of 1mg/ml. F4TCNQ solution was added to cover the surface of the polymer films, left for 20mins for the simultaneous doping process, the doped polymer films were spun again.

2.6.2 pH test

pH testing was done using the RGFET configuration and the change of PH sensitivity was calculated. The pH test started from the acidic regime to the basic regime and the pH sensitivity can be reflected through the change of threshold voltage.

3.Results and discussion

3.1 Morphology of GO-COOH modified PMMA substrate

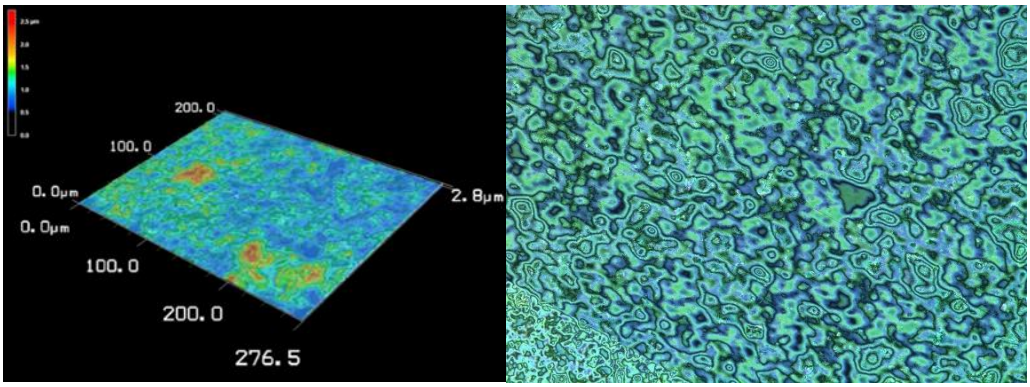


Fig. 3

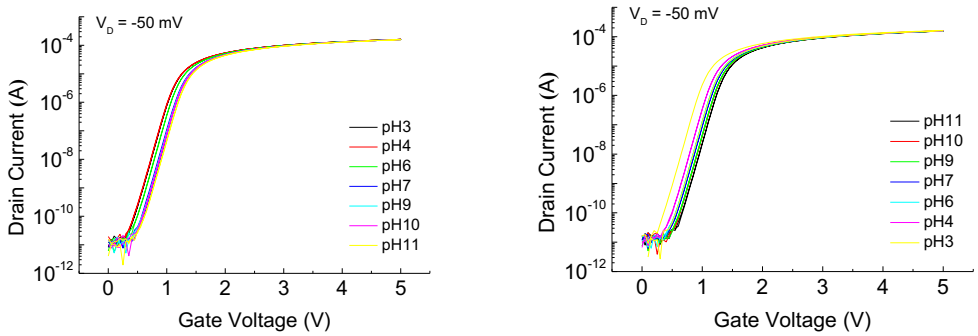
The morphology is shown in Fig 3. GO-COOH is distributed on the PMMA substrate surface. The GO-COOH is distributed on the surface of PMMA, but not evenly. However, after washing by water, unattached GO-COOH can be washed away.

3.2 pH test

The pH test is done on PMMA substrate, GO modified PMMA substrate, GO-COOH modified PMMA substrate and antibody immobilized GO-COOH PMMA substrate.

The measurement is started from pH3 and tested as the following sequence: pH3, pH 4, pH 6, pH 7, pH 9, pH 10, pH 11. The test results are shown below.

The original figure is shown in Fig.4, this is the figure of the part of the test of pure PMMA with ITO on the top. The shift of the threshold voltage is the signal of antibody immobilization, so the threshold voltage is extracted from the original data.



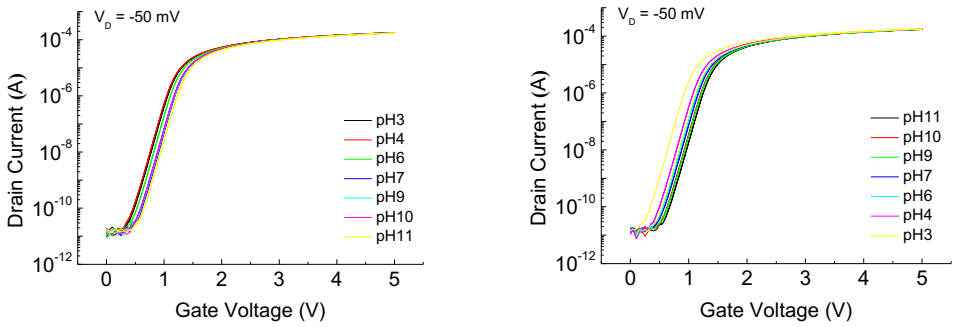


Fig 4.

The extracted threshold voltage can be used and shown in Fig 5.

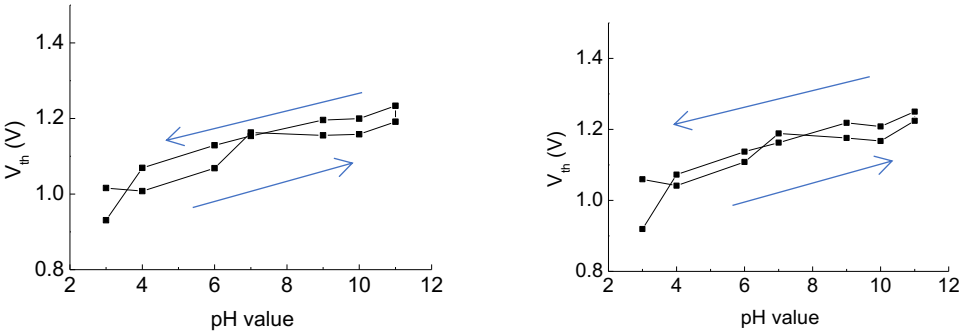
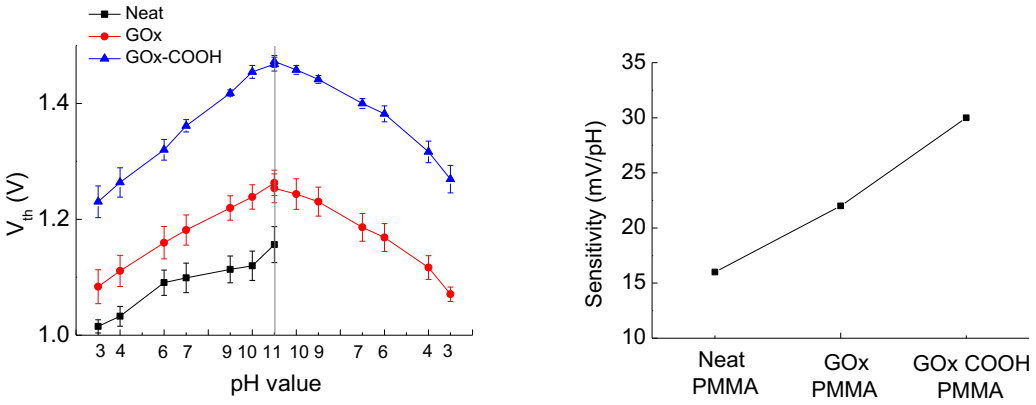
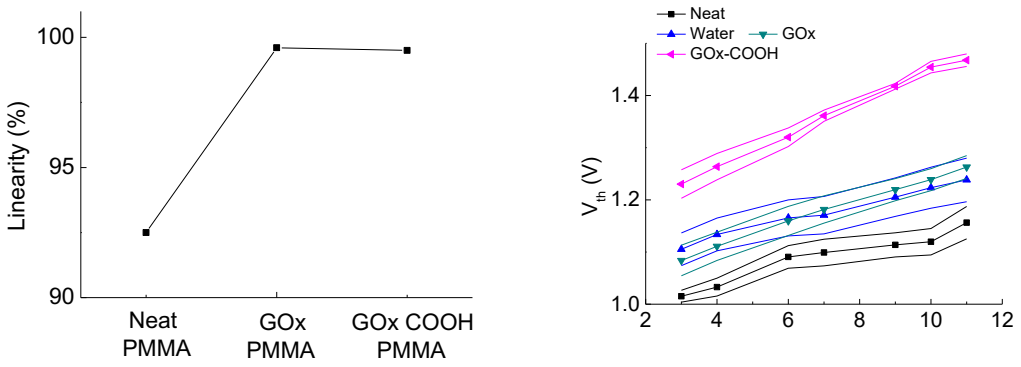


Fig 5.

All the measurements can be portrayed as the change of the threshold voltage.



a) b)



c) d)

Fig 6

Sample	Sensitivity of pH3-11	Linearity	P-value	Sensitivity of pH 11-3	Linearity
Neat XL PMMA 8 samples average	16 mV/pH	92.5 %		Not measured	Not measured
GOx XL PMMA (preheating) 7 samples average	22 mV/pH	99.6%		22 mV/pH	96.3%
GOx COOH XL PMMA 9 samples average	**30 mV/pH**	**99.5%**	**At pH3: 4e-6 At pH7: 8e-8 At pH11: 3e-7**	**25 mV/pH**	**96%**

Table 1.

Fig 6 and Table 1 is the conclusion of the measurement of pure PMMA, GO modified PMMA and GO-COOH modified PMMA. Under the same process of pH sensitivity testing, the threshold voltage and the sensitivity are all different. The increasing sensitivity can reflect the increasing concentration of carboxylic acid groups, indicating GO-COOH can be a good receptor of antibody. This also leads to the next step test, the antibody-antigen reaction test. Control experiments are also done during the process: neat PMMA substrate as well as pure water and GO on PMMA substrate

3.3 antigen test

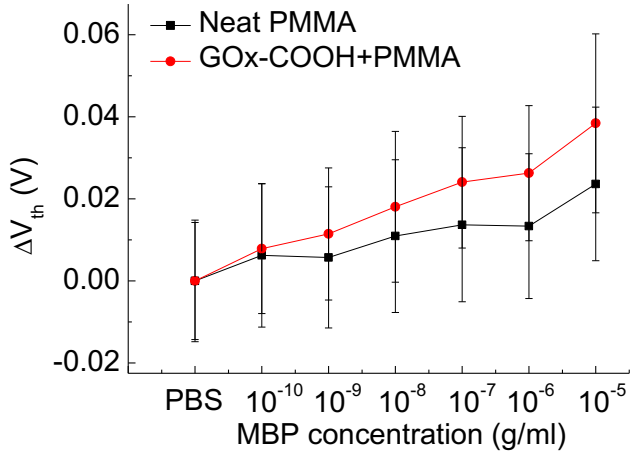
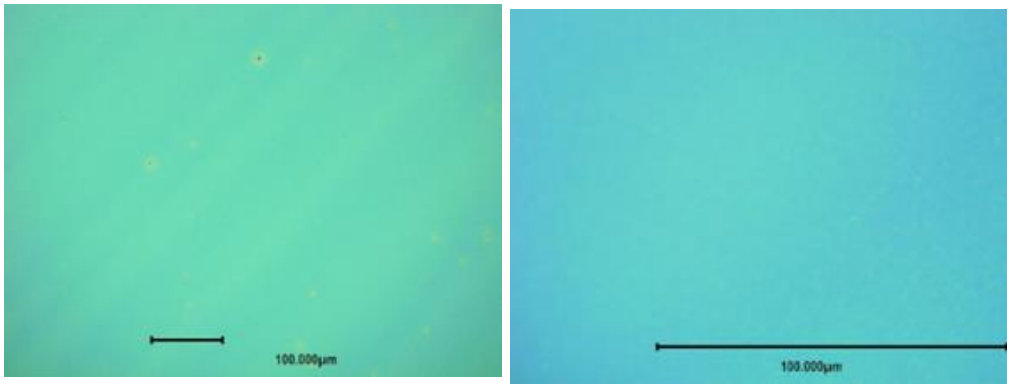


Fig 7

Fig 7 is the output of the antigen test. Neat PMMA and GO-COOH modified PMMA substrate are tested. It is easily observed that GO-COOH modified PMMA has a higher shift of threshold voltage than neat PMMA, which can indicate it has an interaction between antigen and antibody. The higher sensitivity of antigen can be evidence of the immobilization of antibody.

3.4 Morphology of antibody embedded PT-COOH film

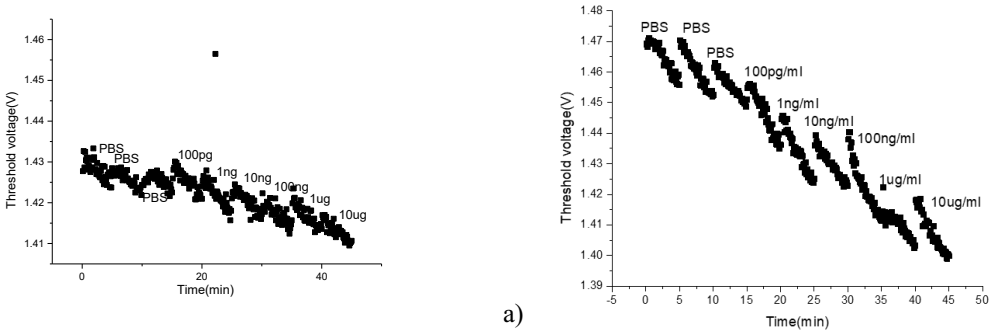


a)20X b)100X

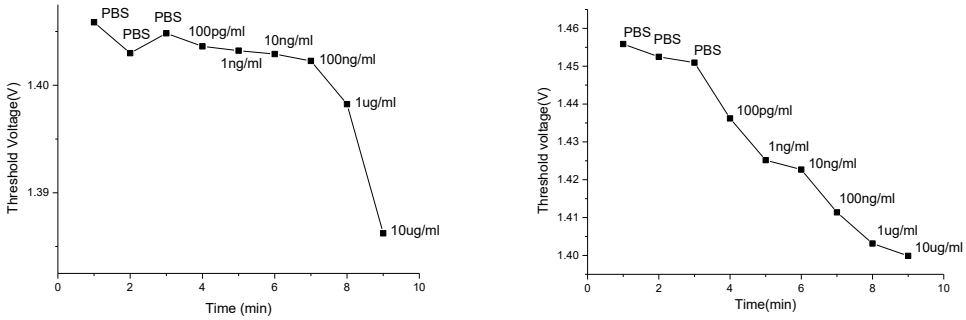
Fig 8

The microscopy image shows that the antibody embedded polymer film has a smooth structure.

3.5 Antigen test



a)



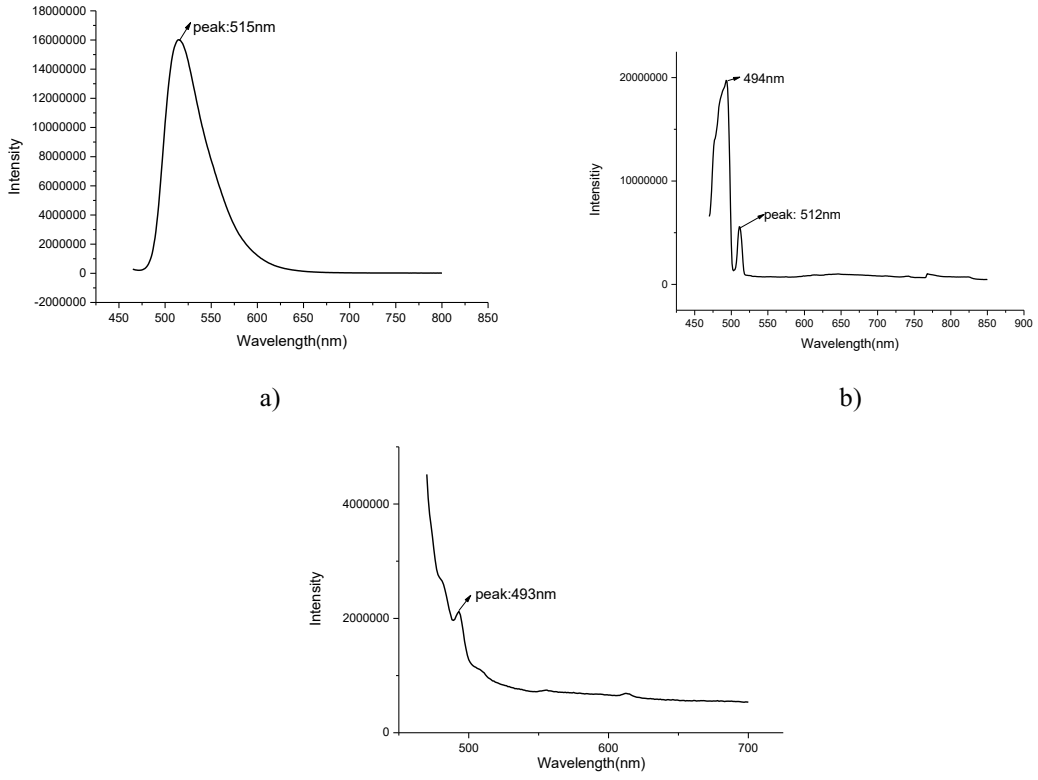
b)

Fig 9

The surface treatment method for antibody binding can shows a signal of threshold voltage change when the antigen test is done. Fig 9 a) is the threshold voltage of all measurements since at every concentration the measurement is repeated twenty times. Fig 9 b) is the threshold voltage of the last measurement. It can be observed that the threshold voltage has a tendency to decrease. However, it is hard to get exactly same data from the same antibody binding and testing process. The small change of the threshold voltage can be caused by the activity of the EDC/NHS solution or the activity

of the polymer films. The antibody is an active biomolecule, so it is also hard to control its degree of binding is exactly the same every time.

3.6 Fluorescence test



a) b)

c)

Fig 10.

Fig 10 a) is the excitation fluorescence spectrum for 10ug/ml antibody solution, Fig 10 b) is the fluorescence spectrum for antibody embedded PT-COOH film and Fig 10 c) is the spectrum for pure PT-COOH film. The concentration of PT-COOH is 20mg/ml and FITC-MBP antibody is 20ug/ml. The peak at 493nm and 494nm can be the cause of Raman scattering during the measurement. The peak at 512nm, which is close to the peak of the antibody solution, is the proof of antibody immobilization. The decrease of fluorescence intensity is because of the quenching of fluorescence or the

low concentration of the antibody. The thiophene rings in PT-COOH can interact with FITC, change it structurally and cause the quenching. The peak is not exactly at 515nm because some antibody can aggregate together and increase the absorption during the bonding and film making process. They cannot distribute so evenly as they can in the solution. Or the antibody can be surrounded by the ions and have some electrostatic interaction with the antibody, which can also affect the peak position.

3.7 Doping effect test

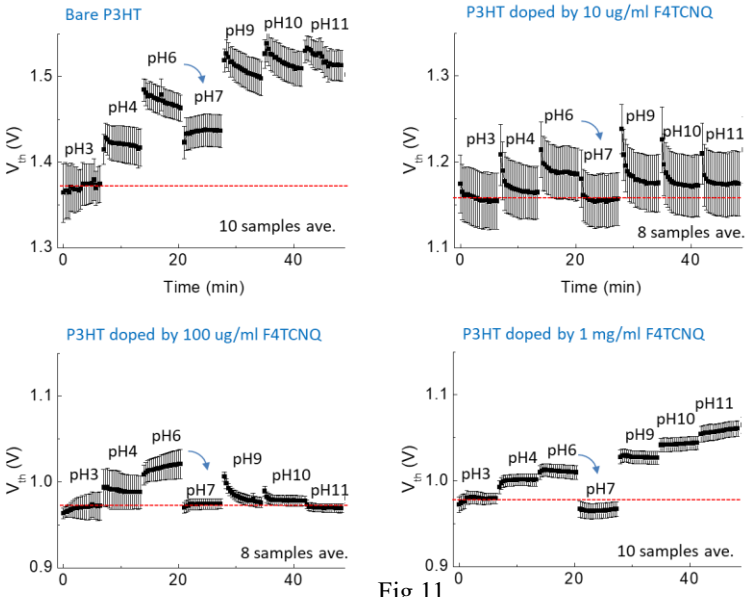


Fig 11

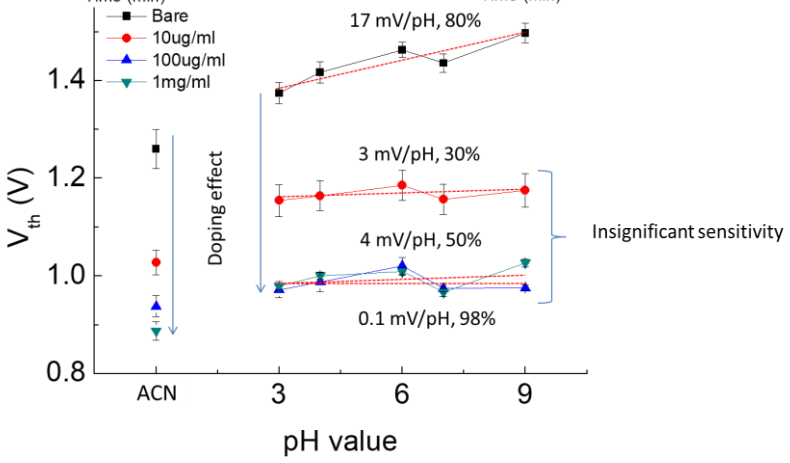


Fig 12

Fig 11 and Fig 12 are the figures for the doping effect test on Poly(3-

hexylthiophene-2,5-diyl) (P3HT), which is a widely used conjugated polymer. It can be easily observed that after doping the pH sensitivity and threshold voltage are both obviously decreased, and with the increase of the concentration of the dopant, the sensitivity decreases more, and the threshold voltage also decreases before the concentration of the dopant reaches 1mg/ml. The data of the test of P3HT indicates the hypothesis that the pH sensitivity is caused by ionic doping is right. P3HT is a p type semiconducting polymer and F4TCNQ increases the concentration of the hole carriers, leading to the increase of positive charge, that is the reason for threshold voltage decrease. The threshold voltage and pH sensitivity are not obviously different between the concentration of 100ug/ml and 1mg/ml because the dopant has possibly reached its saturation point at 100ug/ml, so the increase of dopant concentration will not affect the ionic doping. The dopant may aggregate itself, so the threshold voltage increases a little bit.

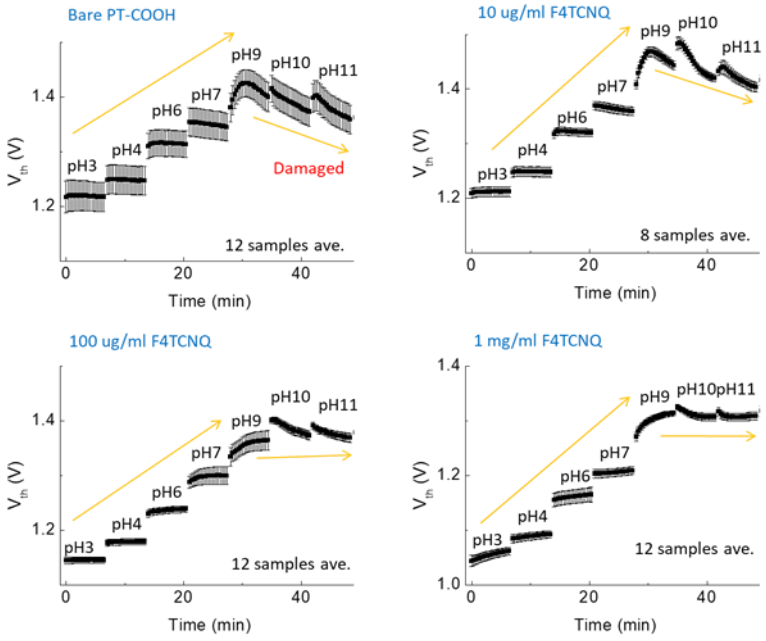


Fig 13

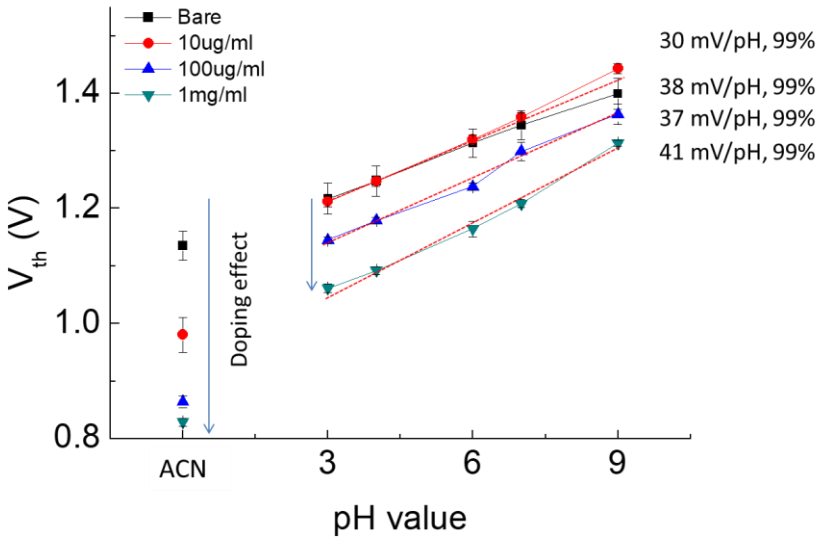


Fig 14

Fig 13 and Fig 14 are the data for PT-COOH doping test. The decrease of threshold voltage is still caused by the increasing concentration of hole carriers. However, the pH sensitivity increases which is the opposite of our hypothesis. This can be caused by the involvement of the carboxylic acid group. The thiophene rings are not the only position that the dopant can interact with. The dopant can also interact with the carboxylic acid groups, which can be the reason for the increasing of sensitivity. The existence of the carboxylic acid group may help create more holes after doping, and that can also increase the sensitivity.

Conclusion

Both oxidized graphene oxide and PT-COOH can be a good receptor for antibody immobilization. The electrical ELISA and fluorescence test indicate that the antibody can bond to these receptors and have interaction with the antigen. These receptors have stable properties, and both can be used in designing biosensors. The doping effect test of P3HT indicates that the pH sensitivity of P3HT is caused by ionic doping, and the doping effect test of PT-COOH indicates that the pH sensitivity caused by ionic doping can be affected by side groups such as carboxylic acid groups. The research that has been done can show that the conjugated polymers and carbon materials can be good materials for biosensing, and P3HT can resist the influence of ions considerably after doping.

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Curriculum Vitae

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Research Interest

- Fabrication of multiple types of carbon materials and conjugated polymers.
- Studies of interfacial properties of sensing materials.
- Fabrication of sensing devices and photovoltaics.

Education Background

09/2018-now **Johns Hopkins University,** Baltimore, Maryland, the US

Current GPA: 3.9/4

Major: Materials Science and Engineering

09/2014-06/2018 **Harbin Institute of Technology (HIT)**, Harbin, Heilongjiang, P.R.China

Major: Polymer Material and Engineering (Bachelor of Engineering)

Total GPA: 3.34/4

Publications

- Cheng, F., Liu C., Li H., Wei X., Yan, T., Wang Y., **Song Y.,** He J., Huang, Y., Carbon nanotubes-modified oxidized regenerated cellulose gauzes for hemostatic applications ***Carbohydrate Polymers,*** 2018, 183:246-253.
- Hyun-June Jang, Justine Wagner, **Yunjia Song**, Taein Lee, Qingyang Zhang, Howard E. Katz (2019), Carboxylic Acid-Functionalized Conjugated Polymer Promoting Diminished Electronic Drift and Amplified Proton Sensitivity of Remote Gates Compared to Nonpolar Surfaces in Aqueous Media, ***Advanced Electronics Materials***, (under review)

Research Experiences

09/2018-now **The Johns Hopkins University**

Supervisor: Prof. Howard Katz Position: Research Assistant

Project 1: Carboxylic Acid-Functionalized Conjugated Polymer Promoting Diminished Electronic Drift and Amplified Proton Sensitivity of Remote Gates Compared to Nonpolar Surfaces in Aqueous Media (completed)

- Sensing materials for a biosensor will inevitably have an interface with solution, under the influence of electric field, the threshold voltage drift of the interface is a common behavior. The purpose of this project is to figure out whether the conjugated polymers have electrochemical stability when they are used as interface sensing materials. The conjugated polymers demonstrate good stability, and they also show proton sensitivity which is a new development. So conjugated polymers can be a good option for chemical sensing.
- Created remote-gate FET (RGFET) configuration by integrating the sensitive surface with a commercial silicon FET.
- After PH7 aqueous buffer solution and organic solvent acetonitrile (ACN) are added on the sensing layers (ITO, SiO_2, PS, PSAA, P3HT and PT-COOH film), the drift of threshold voltage can reflect the surface potential change of them. The drift is caused by dipole orientations at quasi-equilibrium, the extent of drift reflects the electrochemical stability of different materials.
- The proton sensitivity can also be reported through the change of threshold voltage when

PH3-PH11 buffer solution are added on the sensing layers.

Project 2: Studying the relation between doping effect and proton sensitivity

- The hypothesis is that the doping effect of ions is the cause of proton sensitivity.
- Dope the conjugated polymers with dopant and test the proton sensitivity to study the correctness of the hypothesis.

Project 3: Using oxidized graphene oxide /carbon quantum dots modified polymers as sensing layer to fabricate biosensors. (in process)

- Oxidized graphene oxide and carbon quantum dots are materials that have carboxyl group, this kind of materials can bond antibody directly. The specific bonding between antibody and protein can be demonstrated by the drift of threshold voltage. This method can be named as electrical ELISA. Biosensors can be fabricated based on this theory.
- Fabricate oxidized graphene oxide (GO-COOH) and carbon quantum dots.
- Attach GO-COOH on commercially made PMMA substrate by spin-coating and annealing. Mix carbon quantum dots with PSAA solution and make sensing layer through spin-coating
- Test biosensitivity through electrical ELISA.

06/2016- 06/2018 **Harbin Institute of Technology**

Supervisor: Associate Prof. Jinmei He Position: Research Assistant

Project1: Carbon nanotubes (CNTs)-modified oxidized regenerated cellulose (ORC) for hemostatic applications (Completed)

- Modified carbon nanotubes can be more biocompatible and can affect the cell behavior. Therefore, bond CNTs to ORC and test the change of hemostatic property can be a direction to dig into. The consequence showed that CNTs can improve the hemostatic behavior of ORC
- Fabricated the novel ORC composite by grafting unmodified multi-walled carbon nanotubes (MWCNTs), and functionalized carbon nanotubes with amino groups (MWCNTs-NH_2) and carboxyl groups (MWCNTs-COOH).
- Conducted the characterization of physical and chemical properties of MWCNTS/ORC, MWCNTS-NH_2/ORC and MWCNTS-COOH/ORC by XPS, FTIR etc.
- Evaluated the hemostatic properties by animal experiment, using rabbit liver hemostasis model and rabbit ear artery model.

Project 2: Fabrication of nitrogen-doped carbon quantum dots (NCDs) and test the hemostatic (completed)

- Nitrogen-doped carbon quantum dots (NCDs) is a biocompatible material with size of several nanometer, which can be more easily bonded to ORC. It may also improve the hemostatic behavior since it is a kind of carbon material.
- Fabricated nitrogen-doped carbon quantum dots by microwave method and tested the quality of the NCDs by fluorescence spectrum, UV spectrum, and XRD etc.
- Bonded NCDs to ORC and tested hemostatic properties.

Work Experiences

Radiation Transfer Laboratory, Kochi University of Technology, Kami Japan

06/2017-07/2017

Position: research assistant

- Gained the skills of fabrication of ZnO nanorods by CBD (Chemical Bath Deposition) method.
- Gained the skills of films fabrication on substrate by Mist CVD (fine channel) technology.